BENZOQUINOLIZINE-2-CARBOXYLIC ACID ARGININE SALT TETRAHYDRATE

Field of the Invention

[0001] The present invention relates to crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate and to processes for producing it. Compositions incorporating the tetrahydrate to provide formulations for use in the prophylaxis and/or treatment of microbial infectious diseases are also described.

Background of the Invention

[0002] S-(-)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt is a broad-spectrum antibiotic, medically grouped together with the fluoroquinolone class of antibiotics, which is disclosed and claimed in our U.S. Patent 6,514,986 B2 as being isolated in a less crystalline anhydrate form and a more crystalline hydrate form. Our U.S. Patent 6,664,267 describes a crystalline monohydrate form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt that is disclosed as having advantages over the anhydrate and hydrate forms described in U.S. 6,514,986 B2. Such advantageous properties for the crystalline monohydrate form, in comparison to the less crystalline anhydrate and hydrate forms, include enhanced stability at specified conditions of humidity and temperature.

[0003] In accordance with the present invention, it has been found that S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate in highly homogeneous form is advantageous over previously known forms and may be usable to prepare stable pharmaceutical dosage forms, including an aqueous solution, because it is the most physically stable form and does not have a tendency over time to convert to other crystalline forms.

Brief Description of the Drawings

[0004] FIG. 1 shows the single crystal X-ray ORTEP diagram of the of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]

quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

[0005] FIG. 2 shows the hydrogen bonding network of the water molecules in S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

[0006] FIG. 3 is a X-ray Powder Diffraction (XRPD) pattern of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

[0007] FIG. 4 is XRPD spectra illustrating conversion of monohydrate to tetrahydrate.

[0008] FIG. 5 is a Differential Scanning Calorimeter (DSC) analysis of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

[0009] FIG. 6 is a thermogravimetric analysis of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

[0010] FIG. 7 is theoretical XRPD spectrum calculated by a standard software from the coordinates of a single crystal X-ray analysis.

Summary of the Invention

[0011] In accordance with the present invention, there is provided a crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate which is highly homogeneous in regard to other solvatomorphic forms thereof and has superior properties in comparison to such other anhydrate or hydrate solvatomorphic noncrystalline or crystalline forms.

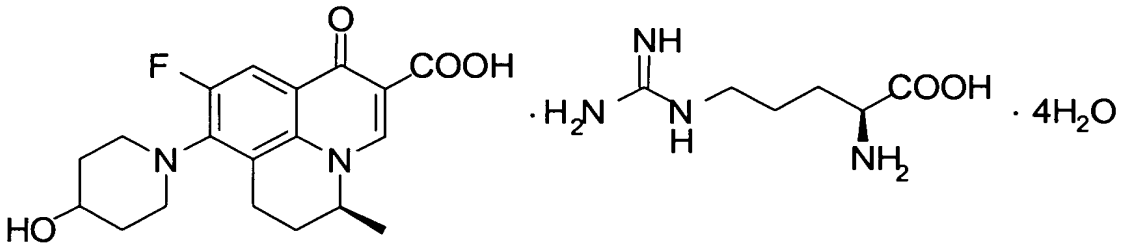
[0012] The present invention further pertains to processes for the preparation of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate, pharmaceutical compositions containing it, and the use thereof in the treatment and/or prevention of a wide variety of microbial infections.

[0013] The S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate of the invention is a compound that shows potent antibacterial activity against gram-positive bacteria, gram-negative bacteria and anaerobic bacteria, as described in more

detail below.

Detailed Description of the Invention

[0014] In accordance with the present invention, there is provided a novel highly homogeneous crystalline tetrahydrate hydratomorphic form of the broad spectrum antibiotic S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H, 5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt, represented by the following structure:



[0015] S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt has been shown in mammals to be effective against a broad spectrum of microorganisms including antibiotic-resistant strains of Staphylococcus aureus, more particularly glycopeptide-resistant staphylococci, and to possess excellent overall tolerability. (Posters F-535, F-537, F-538, F-539, and F-540 presented at 41st ICAAC 2001, Chicago, IL, USA and Poster F-564 presented at 42nd ICAAC 2002, San Diego, CA, USA).

[0016] Initial methods to prepare S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt as a bulk active ingredient resulted in an anhydrate solvatomorphic form and a hydrated solvatomorphic form thereof. The physicochemical characteristics of these solvatomorphs are described in U. S. Patent No. 6,514,986 B2. Solvatomorphism is said to exist when a molecule displays an ability to crystallize in different structures that in turn differ in their solvation state (Brittain, Spectroscopy, (2000), 15 (7), 34-39). A hydratomorph may be defined as a solvatomorph in which the solvent is water. Further investigation in the preparation of bulk material revealed that a crystalline form could be produced of a monohydrate of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt. The physicochemical

characteristics are described in our U.S. Patent 6,664,267. Further investigation of processes to prepare this monohydrate and single crystals of it led to a hydratomorph which on X-ray crystallographic analysis showed it, surprisingly, to be a tetrahydrate, the detailed data for which is provided below and in the illustrations. An in-depth study of the different hydrates of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt in respect of their respective x-ray diffractograms, differential scanning calorimetry graphs, their water content, their stability order as a function of temperature and/or humidity led to an understanding that the tetrahydrate is the most stable polymorph.

[0017] S-(-)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate is further characterized by crystal parameters from single crystal x-ray crystallographic analysis as set forth below.

[0018] The crystal of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt is found to form a molecular assembly having a composition of $C_{19}H_{21}N_2O_4F.C_6H_{14}N_4O_2.4H_2O$; a 1:1 complex of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid and L-arginine with four water molecules of crystallization. The salt exists in the zwitterionic form in the orthorhombic system, space group $P2_12_12_1$. The details of data collection, structure solution and refinement are given in Table 1.

TABLE 1

[0019] Single Crystal Parameters of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

DATA	Compound of the invention
Formula	$C_{19}H_{21}N_2O_4F.C_6H_{14}N_4O_2.4H_2O$
Formula weight	606.64
Temperature/K	293(2)
Wavelength	MoK_α
Crystal system	Orthorhombic
Space Group	$P2_12_12_1$

DATA	Compound of the invention
a/Å	8.475(5)
b/Å	9.378(6)
c/Å	36.753(3)
$\alpha/^{o}$	90.0
$\beta/^{o}$	90.0
$\gamma/^{o}$	90.0
Volume/$Å^3$	2129.2(3)
Z	4
Density/gcm^{-3}	1.38
Abs. Coeff/mm^{-1}	0.111
F(000)	1407.8
$\theta_{min, max}$	2.2, 23.3
$h_{min,max}$, $k_{min,max}$, $l_{min,max}$	-9, 9; -10, 10; -36, 40
No. Refl^{n}. measured	18378
No. unique Reflection	4197
No. parameters	551
Refinement method	Full matrix least square on F^2
R_all	0.062
R_obs	0.041
wR_2 (all)	0.072
wR_2 (obs)	0.067
$\rho_{min,max}/eÅ^{-3}$	-0.132, 0.132
GooF	1.064

[0020] The tests and procedures used to obtain the data included in Table 1 are standard in the art and a person skilled in the art would know how to carry out these tests based on this specification and his/her knowledge of the art.

[0021] The -COOH groups of both S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid and L-arginine are found ionized as $-COO^-$ and H^+. These H^+ ions are taken up by the nitrogen lone pairs of L-arginine to form NH_3^+ and NH_2^+ respectively. The four water molecules were found to form strong hydrogen bonds with the $-COO^-$ groups (Fig.2). In fact, H71 of one of the water molecules forms a strong O-H...O hydrogen

bond with the -COO^- group (H71...O2 in Fig.2) of the benzoquinolizine-2-carboxylic acid, along with yet another strong O...H-N hydrogen bond with -NH_3^+ group of L-arginine (O7...H31 in Fig.2). Thus, this water molecule (O7) bridges the two moieties resulting in a stronger association. However, the other three water molecules, two of which generate a strong O-H...O hydrogen bonds with the -COO^- group of L-arginine (O9H01...O5 and O10H102...06), and the remaining water molecule with –COO^- of the benquinolizine-2-carboxylic acid (O8H81...O2), are less tightly bound, all bindings being shown in Fig.2. A Differential Scanning Calorimetric study (cf. Fig. 5) and Thermogravimetric Analysis (cf. Fig. 6) of the salt of the invention confirms the nature of binding, by three of the four water molecules (O8, O9 and O10) being lost on heating at 70°C, initially generating a monohydrate by retaining O7.

[0022] Crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H, 5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate with the water molecules bound as depicted by the single crystal x-ray analysis may be prepared in high homogeneity by the slow evaporation of the solution made by dissolving S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [i,j] quinolizine-2-carboxylic acid L-arginine salt in an organic solvent and water.

[0023] A process for the manufacture of the crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate comprises the following consecutive steps:

a) heating a suspension of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt in an organic solvent and water at 70°-80°C to obtain a clear solution;

b) cooling the solution to provide a crystalline substance;

c) isolating the crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate at 30°C - 35°C by filtration or centrifugation; and

d) air drying of the crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-

2-carboxylic acid L-arginine salt tetrahydrate at a temperature between 30°C - 35°C.

[0024] Any S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt can be used in step a).

[0025] The preferred organic solvents are acetonitrile and acetone. The most preferred organic solvent is acetone. The preferred ratio of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt to the organic solvent to water is 1:5: 1.25 (w/v/v). The preferred ratio of acetone or acetonitrile to water is (4:1 v/v).

[0026] The solution of step a) is heated for as long as necessary to obtain a clear solution. A clear solution is typically obtained in 15 minutes to 3 hours but the time may vary.

[0027] In step b) the solution may be cooled to room temperature or between 30°C-35°C.

[0028] Of the various crystalline forms of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt, the primary one that crystallizes directly from aqueous acetone solutions is the tetrahydrate. This tetrahydrate shows on Karl Fischer (KF) analysis a water content of 11.87 %, (calculated for $4H_20$: 11.88%), equivalent to four molecules of water of hydration. On further drying for 16 hrs at 70°C and under high vacuum of 1 mm of Hg the tetrahydrate is converted to a monohydrate and analyses for a water content of 4.0 %, (calculated for 1 H_2O: 3.98%).

[0029] This monohydrate is highly unstable and rapidly absorbs moisture under ambient conditions of temperature (35°C) and relative humidity (60 %). Within 20 minutes the XRPD spectra of the monohydrate undergoes changes as illustrated by the diffractograms in Figure 4, wherein the characteristic 2 θ peaks of the monohydrate appearing at 5.28 and 10.66 in Figure 4-A shift to a reduction of the intensity of these peaks in Figure 4-B with a concomitant appearance of peaks at 2 θ values of 4.84 and 39.2 which are characteristic of the tetrahydrate. In Figure 4-C the total disappearance is seen of the peaks of the monohydrate. Its conversion to the tetrahydrate may be confirmed by the increase in the intensity of the 2 θ peaks at 4.84 and 39.42, peaks which overlap with those in Figure 3 obtained for an authentic sample of the tetrahydrate.

[0030] The high homogeneity of the prepared tetrahydrate may be confirmed by comparison of its XRPD spectrum (Fig.3) with that obtained theoretically for single phase tetrahydrate material (Fig.7) by inserting the coordinates derived from a single crystal X-ray structure into a standard software programme. The XRPD spectrum in Fig.3 is seen to be identical with that provided in Fig. 7.

[0031] The water content of the tetrahydrate may range from 11.0 to 12.5% according to the Karl Fischer analysis.

[0032] This novel crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt of this invention is stable under typical storage conditions, has good bioavailability in mammals, has lower phlebitis-forming potential on administration to mammals, has low or reduced toxicity, has acceptable disintegration and dissolution rates, and hence is very useful for pharmaceutical manufacturing and for use in medicine. Formulation of the thermodynamically most stable form is a reasonable expectation for a solution mediated process. Using the most stable form rather than a metastable form is advantageous regarding physical stability of the crystalline form. The increased physical stability will afford additional advantages in formulation. The form is specially suitable for treating diseases caused by microbial infections. The form is suitable for long-term intravenous therapy in critically ill patients or patients in intensive care units. Injectable preparations of the L-arginine salt can be readily prepared in view of its availability in a bulk form that remains stable under specified conditions, its favorable aqueous solubility, its ideal suitability in not causing venous inflammation on repeated intravenous administration, and its safety from adverse toxicity.

[0033] The antibacterial polymorphic compound of the invention is useful in the treatment of mammals having a broad spectrum of bacterial infections such as impetigo, pneumonia, bronchitis, pharyngitis, endocarditis, urinary tract infections, gastro-intestinal infections and bacteremias caused by Staphylococcus aureus, coagulase negative staphylococci, methicillin-resitant Staphylococcus aureus, methicillin-resitant coagulase negative staphylococci, enterococci, beta-haemolytic streptococci, viridans group of streptococci, mycobacterial infections due to multi-drug resistant M. tuberculosis and other atypical mycobacteria such as M. intracellulare and M. avium, as well as newly emerging Gram-negative pathogens such as Chryseobacterium meningosepticum, Chryseobacterium indologense and other Gram-negative pathogens

such as E.coli, Klebsiella, Proteus, Serratia, Citrobacter, and Pseudomonas.

[0034] The present invention also encompasses an antiinfective composition for the treatment of humans and animals in need of prophylaxis and/or therapy for systemic or topical infections especially resistant gram-positive organism infections, gram-negative organism infections, mycobacterial infections and nosocomial pathogen infections, which composition comprises an amount of the compound of the invention substantially sufficient to eradicate said infection, but not to cause any undue side effects. The compound and compositions of this invention can be administered to humans and animals who are at risk of being infected, for example a compound or composition of this invention can be administered to a patient prior to and/or after surgery, health care workers or others who are at risk of being infected.

[0035] These findings have an important implication from the point of view of the systemic use of the compound of the invention in view of its superior potency, superior bactericidal activity, expanded biospectrum, better bioavailability and improved tolerability which are now enabled to be administered systemically in therapeutically effective doses. Utilizing the compound of the invention, whether in systemic or topical dosage form, results in clearer dose-related definitions of efficacy, diminished toxic effects and accordingly an improved therapeutic index.

[0036] The present invention encompasses the compound of administering the compounds to a human or other animal subject. The compound and compositions to be used in the invention must, accordingly, be pharmaceutically acceptable. As used herein, such a "pharmaceutically acceptable" component is one that is suitable for use with humans and / or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

[0037] The pharmaceutical compositions are prepared according to conventional procedures used by persons skilled in the art to make stable and effective compositions. Such methods include combining the tetrahydrate of this invention with suitable a carrier, diluent, solvent or excipient. In the solid, liquid, parenteral and topical dosage forms, an effective amount of the active compound or the active ingredient is any amount, which produces the desired results.

[0038] It has been found in accordance with the present invention that the advantageous stability and solubility properties of the tetrahydrate of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt can be applied to the formulation of

pharmaceutical dosage forms. Tables providing stability and solubility data are included in the examples. The tetrahydrate of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt can be used to prepare aqueous dosage forms. It can also be used to prepare tablets by wet granulation; it can also be formulated by conventional dry granulation.

[0039] The dosage forms can be prepared by any conventional techniques recognized in the art, but would preferably be formulated by mixing the tetrahydrate salt of the invention with the other ingredients. The other ingredients utilized to formulate solid oral dosage forms would include conventional inert ingredients such as microcrystalline cellulose, methyl cellulose and the like, suitable sweetening and/or flavouring agents, and preservatives thereof if required.

[0040] Such solid oral dosage forms or dry formulations suitable for the preparation of suspensions would be formulated such that they would contain an effective dose of the compound of the invention. In general, solid dosage forms containing 100 mg - 1500 mg of the compound of the invention are contemplated. Preparations suitable for oral suspension would contain a similar dosage.

[0041] Pharmaceutical formulations can be formulated together with auxiliaries and additives usually employed in pharmacy, such as tablet binders, fillers, preservatives, tablet disintegrating agents, flow regulating, agents, plasticizers, wetting agents, dispersing agents, emulsifiers, solvents, pH altering additives, flavourings and the like. A second preferred method is parenterally for intramuscular, intravenous or subcutaneous administration.

[0042] When the pharmaceutical composition is formulated into an injectable preparation, in formulating the pharmaceutical composition into the form of a solution or suspension, all diluents customarily used in the art can be used. Examples of suitable diluents are water, ethyl alcohol, polypropylene glycol, ethoxylated isostearyl alcohol, polyoxyethylene sorbitol, and sorbitan esters. Sodium chloride, glucose or glycerol may be incorporated into a therapeutic agent.

[0043] It is preferred that the concentration of active ingredient in the injectable preparation be in the range of 0.1 mg/ml to 100 mg/ml.

[0044] A third preferred route of administration is topically, for which creams, ointments, sprays, shampoos, lotions, gels, dusting powders and the like are well suited.

Generally, an effective amount of the compound according to this invention in a topical form 0.1% composition is to about 10% by weight of the total composition. Preferably, the effective amount is 1% of the total composition.

[0045] For topical application, there are employed as non-sprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g. preservatives, antioxidants, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient preferably in combination with a solid or liquid inert carrier material.

[0046] In addition to the common dosage forms set out above, the compounds of the present invention may also be administered by controlled release means and/or delivery devices such as those described in U. S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123 and 4,008,719; the disclosures of which are hereby incorporated by reference.

[0047] The total daily dose range is generally from about 200 mg to about 1500 mg of the arginine salt form. However, the dose may be higher or lower depending on the needs and conditions of the patient.

[0048] The following detailed examples serve to more fully illustrate the invention without limiting its scope. It is understood that various other embodiments and modifications in the practice of the invention will be apparent to, and can be readily made by, those or ordinary skill in the art without departing from the scope and spirit of the invention as described above. Accordingly, it is not intended that the scope of the claims appended hereto be limited to the exact description set forth above, but rather than the claims be construed as encompassing all of the features of patentable novelty that reside in the present invention, including all of the features and embodiments that would be treated as equivalents thereof by those skilled in the relevant art. The invention is further described with reference to the following experimental work.

EXAMPLE 1

Preparation of the single crystal of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt terahydrate

[0049] S-(-)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt (1.0 g) was dissolved in a mixture of acetone (40 ml) and water (10 ml) by heating the suspension at 70 ^{0}C for 15 minutes. The clear solution thus obtained was left for slow evaporation at room temperature in a beaker covered with a perforated aluminum foil. The crystal formation started after 2 days. Finally the single crystal was selected for X–ray crystal analysis from a cluster left after complete evaporation of the solvent. The ORTEP diagrams are described in Figures 1 and 2.

EXAMPLE 2

Larger scale preparation of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt terahydrate

[0050] To a three-necked round bottom flask fitted on oil bath and equipped with magnetic stirrer and reflux condenser; S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt (20.0 gm, 55.55 mmoles) was charged in a mixture of acetone (100 ml) and water (25 ml). The reaction mixture was slowly heated under stirring at 70°C temperature to obtain a clear solution. The solution was allowed to cool to 30°C - 35°C, a crystalline solid obtained was filtered and air dried at a temperature between 30°C - 35°C to afford the title compound 23.5 gm, (80 %). The moisture content by Karl Fisher titration was found to be 11.87% (required for tetrahydrate: 11.88%). The XRPD, DSC, TGA data were determined as described in the Test Examples provided below. The results obtained are described in Figures 3, 5, 6.

TEST EXAMPLE 1

Single crystal X-ray analysis

[0051] The room temperature single crystal X-ray diffraction data on a prism shaped single crystal were collected on a Bruker AXS single crystal X-ray diffractometer using SMART APEX CCD detector at room temperature [293(2)°K]. The X-ray generator was operated at 50 KV and 35 mA using MoK_α radiation. Data were collected with a ω scan width of 0.3°. A total of 606 frames per sets were collected in three different settings of φ (0°, 90° and 180°) keeping the sample to detector distance of 6.03

cm and the 2θ-value fixed at -25°. The data were reduced by SAINTPLUS [Bruker. SMART, SAINT, SADABS, XPREP, SHELXTL. Bruker AXS Inc. Madison. Wisconsin, USA. 1998] and an empirical absorption correction was applied using the package SADABS [Bruker. SMART, SAINT, SADABS, XPREP, SHELXTL. Bruker AXS Inc. Madison. Wisconsin, USA. 1998]. All the structures were solved using SIR92 [Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. SIR92 - A program for crystal structure solution. J. Appl. Crystallogr. 1993, 26, 343.] and refined using SHELXL97 [Sheldrick G. M. SHELXL97, Program for crystal structure refinement, University of Göttingen, Germany. 1997.].

[0052] Molecular and packing diagrams were generated by ORTEP32 [Farrugia, L. J. J. Appl. Cryst. 1997, 30, 565.] and CAMERON [Watkin, D. M.; Pearce, L.; Prout, C. K. CAMERON - A Molecular Graphics Package. Chemical Crystallography Laboratory, University of Oxford, England. 1993.] present in the WINGX (Version 1.64.03b) [Farrugia, L. J. WINGX. J. Appl. Cryst. 1999, 32, 837.] program suite. The geometric calculations were done by PARST95 [Nardelli, M. J. Appl. Cryst. 1995, 28, 569.] and PLATON97 [Spek, A. L. Acta Crystallogr. Sect A 1990, 46, C34.].

[0053] The ORTEP diagram of the single crystal (Fig. 1) shows the four water molecules.

[0054] The product was analysed for moisture content (12.37 %) by KF titration.

TEST EXAMPLE 2

Powder X-ray Diffraction Analysis

[0055] 300 mg of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [I,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate prepared as in Example 1 was thinly spread on a sample holder. X-ray diffraction analyses (40kv x 40 mA Rigaku D/max 2200) were performed under the conditions listed below:

Scan speed 5°/ min or 20°/ min

Sampling time 7 min or 2 min

Scan mode: continuous

2θ/θ reflection

Cu target (Ni filter)

[0056] The X-ray powder diffraction (XRPD) spectra of the title compound is shown in Fig. 3.

[0057] (2θ):4.86± 0.2,14.10 ± 0.2, 14.90 ± 0.2, 19.35 ± 0.2, 22.20 ± 0.2, 23.04 ±

0.2, 23.54 ± 0.2, 28.44 ± 0.2, 39.44 ± 0.2.

[0058] The X-ray powder diffraction (XRPD) spectra of the monohydrate, the tetrahydrate and a mixture thereof is shown in Fig. 4.

TEST EXAMPLE 3

Differential Scanning Calorimetry

[0059] The Differential Scanning Calorimetry (DSC) was recorded on METTLER TOLEDO STAR system. 5 to 6 mg of the sample was weighed into the aluminum pan, which was then press sealed with an aluminium lid. After three tiny needle holes were made on the lid the sample was tested by heating from 30°C to 300°C at a rate of 10°C/min.

[0060] The differential scanning calorimetry (DSC) analysis of the title compound is shown in Fig. 5. A DSC exotherm at 194.93°C (onset at 189.42°C) and one endotherm at 87.83°C, 144.03°C and 251.26°C

Test Example 4

Thermogravimetric analysis

[0061] Thermogravimetric Analysis (TGA) was recorded on a METTLER TOLEDO TGA/SDTA 851 system. 5 to 10 mg of the sample was weighed into the aluminum pan and sample was tested by heating from 30°C to 300°C at a rate of 10°C/min.

[0062] The thermogravimetric analysis (TGA) of the title compound is shown in Fig 6.

Test Example 5

pH-Related solublility of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [I,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate at 30°C

[0063] An accurately weighed amount about 20 mg of the compound was transferred to a conical flask and a buffer solution of appropriate pH was added in portions (0.2 ml at a time) till a clear solution was obtained. At buffer pH values of 8.0 and 8.5 addition of buffer was discontinued at 20 ml.

pH of buffer	Solubility (mg/ml)
8.0	< 1.0
8.5	< 1.0
9.0	2.0
9.5	5.0

Test Example 6

Temperature/Relative Humidity-related stability of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [I,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate

[0064] 3 Sample batches of the compound were stored under the conditions of the study as stated in the table below. The assay was done by a validated HPLC method. The results are provided in the table below.

Temp.(°C) /Relative Humidity (%)	Time (months)			
	Initial	1	2	3
40 / 75	99.31 %	99.30 %	99.25%	99.27%
25 / 60	99.31%	-	-	99.22%

[0065] The crystallinity of the substance underwent no changes as seen by XRPD spectra.